Original Article

DOI: 10.5582/ddt.2022.01119

Skin properties of itching without symptoms and associated factors among older adults in long-term care facilities

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SUMMARY Since itching without rash frequently among older adults' population, study about skin properties of itching without rash is important to develop prevention methods. Therefore, this study explored the skin properties related to itching without rash and the factors associated with them. A correlation, predictive designs study was conducted at Indonesian Long-term Care (LTC) facilities. Skin properties including skin barrier function and skin inflammation were examined by photographs (macroscopic and microscopic), stratum corneum (SC) hydration, skin Potential of Hydrogen (pH), and skin blotting. Itching experience and skincare behavior were obtained by questionnaire. The itching-related skin properties and associated factors were analyzed. A total of 405 residents participated in this study, with mean age was 74 years. The prevalence of itching on the whole body was 69.1%, and 50.3% of those manifesting itching on the left forearm involved itching without macroscopic abnormalities (itching without rash). SC hydration, skin pH, albumin and nerve growth factor β (NGFβ) were associated with itching without rash (p = 0.007, 0.012, < 0.001, and < 0.001, respectively). Additionally, factors associated with skin properties were age, sex, sun exposure experience, skincare, and hygiene care in the linear regression analysis. Measurement of skin biomarkers using skin blotting was a possible objective measurement of itching skin properties without rash regardless of the environmental condition.

Keywords Health service, hygiene care, health care quality, itching, long-term care, prevalence

1. Introduction

Itching is a predominant skin symptom in older adults (*1-3*). While these adults who suffer from itching can express the need for treatment and skincare, those with cognitive impairment have difficulty expressing their discomfort. Health workers in long-term care (LTC) must detect itching from the skin appearance in such cases. Itching can be due to dermatological diseases (*4*) but may also occur without macroscopic skin abnormalities, making them difficult to be assessed in bedridden patients.

Appropriate treatment requires valid skin examination (*5*). There is an objective skin assessment technique to measure skin properties (*6*). A study on the validity of skin blotting to test for the presence of itchy skin found that albumin, nerve growth factor β (NGFβ), and thymic stromal lymphopoietin (TSLP) are associated with itching (*7*). However, the association of these skin blotting biomarkers with itchy skin without rash needs to be examined.

Early detection of skin conditions will help health workers to cure and prevent severe itching (*8*). Knowledge of the factors associated with the skin properties of itchy skin without rash is also important for planning preventative measures. Therefore, we conducted a dermato-epidemiological study of itchy skin to determine measures to prevent itching.

As the triggers of itching differ among inflammation types, we planned to separately assess itching without rash-associated factors of each inflammation type and skin barrier functionality to identify intervention targets

for preventing itching without rash. Previous studies have reported an association between itching and factors such as age, sweat, sun exposure, season, and systemic disease (*9-12*). However, existing studies have focused on the general population and general itching but did not examine the factors associated with the properties of itchy skin.

This study aims to examine the properties and associated factors of itchy skin without rash, including skin barrier function and inflammation status, in older adults at LTC facilities in Indonesia.

2. Materials and Methods

2.1. Study design and setting

This study used a correlative, predictive design. It clarified itching without rash among the older adult population (prevalence, skin properties, and factors related to those skin properties). The participant was older adults who stayed at three LTCs in Indonesia between January and September 2016. The current study was analyzed and reported after we confirmed the validity of skin blotting examination to detect itching on the skin of older adults (*7*). These facilities provide accommodation for physically and cognitively relative normal older adults and are funded by the government.

2.2. Study participants

All residents of the three LTCs constituted the study population. For sample size, we performed a power analysis based on the effect size of itchy skin in the preliminary study (alpha = 0.05, power = 0.80), along with the number of study variables. We recruited all older adults at the three LTCs to account for attrition. The inclusion criterion was age ≥ 65 years. The exclusion criteria were difficulty in communicating; clinically inappropriate (*e.g.*, severe illness, total paralysis); memory difficulties; and participation in the preliminary study. A preliminary study was conducted among 35 older adults to confirm the research protocols, tools, and skin examinations.

All participants received an explanation of the study. Participants were told not to bathe or perform ablution 30 minutes before the skin examination and not to use skincare/ointment products on that day. Residents in the LTC receive equal daily care, while residents who need medical treatment are transferred to the hospital.

2.3. Measurements

Measurement was conducted using the cross-sectional method. Data collection was performed by certified wound, ostomy, and continence nurse researchers. The nurse researcher was trained to perform skin blotting and other skin examinations.

The study protocol was approved by the Research Ethics Committee of the Graduate School of Medicine, the University of Tokyo (11076). All procedures followed the Declaration of Helsinki. The presence of adverse events of skin examination was confirmed the day after. The principal investigator conducted the interviews and skin measurements. The researcher explained the study protocol and obtained written informed consent from all participants.

2.4. Investigation of itching

Information regarding itching was collected through a questionnaire based on the guidelines of the International Forum for the Study of Itch (*13*). We defined itching as the sensation of needing to scratch the skin. The questionnaire included the body parts affected (forearm, back, thighs, chest, legs, abdomen, instep, fingers, palm, groin), frequency of itching (daily, weekly, monthly), length/duration of daily itching (< 6, 6-12, and > 12 h/day), intensity (mild, moderate, and severe), sensation of itching (pure itching, stinging, burning, mixed sensation), and scratch response to itching (rubbing, squeezing, skin pinching).

2.5. Classification of itching

The dermatologist diagnosed the skin disease based on the interview data and skin photographs (macroscopic and microscopic). In patients with itching, clinical signs such as scaly areas, inflammation, swelling, rash, leathery patches, crusting, visible sweat ducts, and dark colored patches of skin were assessed. The dermatologist defined normal skin, itching without rash, or other skin diseases such as xerosis, prurigo, miliaria, or eczema. The term "itching without rash" was used when participants who complained of itchy skin but had no skin signs while the term "normal skin" was used when the participants had no clinical signs or itching symptoms.

2.6. Skin properties

Skin property examinations, including macroscopic and microscopic examination, stratum corneum (SC) hydration and skin Potential of Hydrogen (pH) measurement, and skin blotting, were performed to evaluate skin inflammation and barrier function. We examined the skin on the outer side of the left forearm between the elbow and wrist. The selection of skin examination site was based on the skin blotting reliability and validity in a previous studies (*7*,*14*).

Details of the skin examination procedure have been described elsewhere (*7*). Briefly, macroscopic examination using a digital camera (Samsung, Seoul, KOR) and microscopic examination (3R Systems, Fukuoka, JP) were performed. Photographs were taken three times for each forearm and included color charts.

From the photos and participants' itch symptoms, a dermatologist and certified wound, ostomy, and continence nurse researcher made the diagnoses. The participant data were blinded to the skin blotting results, without names. We diagnosed the skin for itch without abnormal skin appearance or with abnormalities such as dry skin, eczema, miliaria, or prurigo.

After skin photography, we measured SC hydration and skin pH, SC hydration was measured using mobile skin moisture (Courage+Khazaka Electronic GmbH, Cologne, DE) and skin pH using Skincheck1 (Hanna Instruments, Woonsocket, RI). These measurements were performed three times, and the mean of those examinations was used. The mobile skin moisture was attached to the measurement site until a value appeared on the screen. Higher SC hydration levels indicated good skin moisture and skin barrier function (*15*). To measure skin pH, the Skincheck^{TM}1 was attached to the measurement site after dipping in a neutral solution. The skin pH value is displayed on the screen. A natural skin surface was indicated by lower skin pH (4.7-< 5) (*16*).

For skin blotting, the biomarkers were albumin for skin barrier function (*17*), nerve growth factor β (NGFβ) for epidermal inflammation (*18*), interleukin (IL) 2 for Th1 inflammation (*19*) and TSLP for Th2 inflammation (*20*). The presence of those biomarkers on the blotting membrane has been validated previously (*7,14,17,21,22*). We confirmed the validity of skin blotting for measuring albumin and NGFβ to detect itching of the skin in older adults (*7*).

Skin blotting was developed as an objective skin assessment technique to measure skin biomarkers (*6*). To prepare the skin blotting kit, a piece of 1-cm square nitrocellulose membrane (Bio-Rad Inc., Hercules, CA) was attached with filter paper, gentle medical adhesive tape (Nitoms, Inc., Tokyo, JP), and adhesive tape. To attach the skin blot to the skin, a skin blotting kit pre-wetted with 50 µL of normal saline was attached with medical tape to the measurement site for 10 min. Participants were asked to limit movement of the measurement site. Thereafter, the blot was removed from the skin with a minimal stimulus. Then skin blotting kit was attached to filter paper. The collected membranes were kept dry and stored at 4°C until staining (*6*).

For skin blotting analysis, we analyzed biomarkers for albumin, NGFβ, IL2, and TSLP. Skin blotting membrane was divided into two pieces, and each piece was subjected to immunological double staining according the host of antibodies. Therefore, we performed double staining for albumin (American Qualex Inc., San Clemente, CA) and NGFβ (Abcam Plc., Cambridge, UK) or TSLP (R&D Systems, Minneapolis, MN) and IL2 (Cell Signaling Technology, Inc., Danvers, MA) after blocking using Blocking One (Nacalai Tesque, Kyoto, JP).

Before staining, we confirmed the antibody reaction's reactivity, specificity, and linearity using dot blot and western blot samples. We used different amounts (0.2-200 ng) of full-length recombinant proteins for dot blot examination and cell lysate or tissue homogenate, including the target proteins, for western blot examination. We used only those antibodies whose specificity and dose-dependent immunoreactivity was confirmed in these examinations. Direct method using appropriate secondary antibodies labelled with peroxidase was used to detect albumin, while NGFβ, IL2, and TSLP using indirect method.

We used SNAP i.d. 2.0 Protein Detection System (Merck Millipore, Billerica, MA) for immunostaining procedures. Then, immunoreactivity was visualized using chemiluminescent substrates for alkaline phosphatase (BioFX Laboratories, Owings Mills, MD) and peroxidase (Merck Millipore, Billerica, MA). Thereafter, a chemiluminescence imaging system (Liponics Inc., Tokyo, JP) was used to capture the signal. All process of immunostaining was conducted by a professional laboratory staff.

Biomarker levels on the skin blot were quantified using ImageJ image analysis software (National Institutes of Health, Bethesda, MD). ImageJ separated the pictures into red, green, blue (RGB) color images. Then, the overlaying of corresponding bright field images by GNU Image Manipulation Program 2.6.5 was used to measure the area. The level of biomarker protein secretion was identified based on the signal intensity level on the skin blotting membrane using ImageJ. Skin blotting pictures were normalized with that of the positive control.

2.7. Participant characteristics and skincare behaviors

These were obtained through the questionnaires and medical records. Participant characteristics included age, sex, body mass index (BMI), literacy, cumulative lifetime sun exposure at work, and activities of daily living (ADL) using the Barthel Index (*23*,*24*). The cumulative lifetime sun exposure at work was calculated from the average daily sun exposure (hours) across 365 days for the total years of work (*25*).

Skincare behavior (current and past) included bathing frequency and duration, body washing while bathing, clothing change frequency, and clothing type and material. Data on prevalence and experience of itching were collected using a questionnaire based on the International Forum for the Study of Itching guideline (*13*).

2.8. Statistical analysis

The prevalence of itching was described using frequency distributions and measures of central tendency. To confirm the skin properties of itchy skin without rash, we classified participants into 'itching without rash' and 'normal skin' groups. The Mann-Whitney *U* test was

used to compare skin properties between the groups. The Kruskal-Wallis and Mann-Whitney *U* tests were used to measure the association between participant characteristics and skin properties for categorical and continuous variables, respectively.

To predict the associated factors of skin properties of itching without rash, multiple linear regression analyses were performed using skin properties as the dependent variable. Variables were simultaneously entered in the models based on the literature review; variables with $p \leq 0.05$ in univariate analysis were considered significant. A multicollinearity test was conducted before the variables were entered in the models, and the variance inflation factor was calculated. All analyses were performed using SPSS ver. 26 for Windows (IBM corp., Armonk, USA), and $p < 0.05$ was considered statistically significant.

3. Results

3.1. Participant characteristics and skincare behaviors

A total of 469 eligible residents were available in LTCs. We excluded 64 older adults because of severe psychological conditions, hospitalization, communication disorders, an inability to speak Indonesian or Java, and participation in a preliminary study. Of the remaining 405 residents (response rate = 96.5%), 14 declined to participate, so 391 participants were included.

As shown in Table 1, the mean age was 74 years and 61.6% were female. The proportion of independent older adults was 66.8%. For skincare behavior, 30.2% of participants had a skincare routine before moving into the LTC, with only 10.2% continuing thereafter. Overall, 49.9% bathed ≥ 2 times/day, while 63.9% changed their clothes ≤ 1 time/day, implying that they wore the same clothing during the day and night.

For the prevalence of itching, 69.1% had itching symptoms, which occurred mainly on the forearms (43.7%), back (40.7%), and thighs (34.8%). Older adults who reported forearm itching (n = 171) experienced it daily (95.9%).

Table 1. Participant characteristics (n = 391)

Items	Mean ± SD or n (%)
Demographic factors	
Age (years)	74.3 ± 7.3
Sex (male)	241 (61.6)
BMI (kg/m^2)	21.2 ± 3.3
Barthel Index, (independent)	261 (66.8)
Literate (yes)	172 (44.0)
Cumulative lifetime sun exposure at work (hours)	79.210.1 ± 28.072.4
Skincare behaviors	
Past skincare regimen (yes)	118 (30.2)
Current skincare regimen (yes)	40 (10.2)
Bathing frequency (≥ 2 times/day)	195 (49.9)
Bathing duration (≥ 5 minutes)	299 (76.5)
Wash body while bathing (yes)	308 (78.7)
Clothing change frequency (≤ 1 time/day)	250 (63.9)
Clothing type (short sleeves)	344 (88.0)
Clothing material (cotton)	377 (96.4)
Itching experience*	
Itching perception on any part of body (yes)[†]:	270 (69.1)
Forearm	171 (43.7)
Back	159 (40.7)
Thighs	136 (34.8)
Chest	126 (32.2)
Legs	114 (29.2)
Abdomen	110 (28.1)
Instep	102 (26.1)
Fingers	99 (25.3)
Palm	101 (25.8)
Groin	85 (21.7)
Experience of left forearm itch [‡] (n = 171)	
Frequency (daily)	164 (95.9)
Duration of symptoms (> 12 h/day)	69 (40.4)
Intensity (moderate)	87 (50.9)
Sensory (pure itching)	155 (90.6)
Scratch response (rubbing)	169 (98.8)

BMI, Body Mass Index; SD, Standard deviation. * One participant had more than one itchy skin area. [†]Prevalence of itching in older adults. [‡]The complete skin examination was conducted on the left forearm: Frequency (daily, weekly, monthly), Duration of symptoms (< 6 h/d, 6-12 h/d, > 12 h/d), Intensity (mild, moderate, severe), Sensory (pure itching, stinging, burning, mixed sensation), Scratch response (rubbing, squeezing, pinching the skin).

Table 2. Skin diagnosis on left forearm by a dermatologist*

Items	Itching symptoms n (%)	
	No (n = 226)	Yes (n = 165)
Normal skin	193 (85.4)	0 (0)
Itching without rash	0 (0)	83 (50.3)
Xerosis	17 (7.5)	30 (18.2)
Eczema	4 (1.8)	44 (26.7)
Others[†]	12 (5.3)	8 (4.8)

* The dermatologist diagnosed the skin on the left forearm based on skin photographs (macroscopic and microscopic) and itching symptoms. For example, a participant had itching on their left forearm without skin signs. A dermatologist then diagnosed 'itching without rash'. [†]Other skin diseases: prurigo, miliaria, dry skin.

3.2. Skin diagnosis and itching symptoms

Table 2 shows the left forearm diagnosis by a dermatologist. There were 193 participants who did not report itching and were diagnosed as having normal skin on forearm (Figure 1a). Of the 165 participants who reported left forearm itching, 50.3% were diagnosed with itching without rash on forearm (Figure 1b) and 49.7% with other skin diseases because itching was accompanied by clinical signs on forearm (Figure 1c).

3.3. Itching-related skin properties

To identify the specific skin properties associated with itching without rash, we compared the properties between participants who had itching without rash and those with normal skin (Table 3). We excluded skin with other diseases (n = 115). Participants with itching without rash had significantly higher skin pH, albumin,

and NGFβ (p = 0.012, < 0.001, < 0.001, respectively), and significantly lower SC hydration (p = 0.007) than those with normal skin.

3.4. Factors associated with skin properties of itching without rash

Differences in SC hydration, skin pH, and skin blotting findings based on the participants' characteristics were analyzed using univariate analysis (data not shown). The following associations were observed: SC hydration with past skin care (p = 0.001), bathing frequency (p = 0.002), bathing duration (p = 0.035), clothing change frequency (p = 0.038), and clothing type (p = 0.011); skin pH with past skin care (p = 0.018), bathing frequency (p = 0.004), and clothing change frequency (p = 0.031); albumin level with cumulative lifetime sun exposure at work (p = 0.046), past skin care (p < 0.001), bathing frequency (p < 0.001), and clothing type (p = 0.002); and NGFβ level with cumulative lifetime sun exposure at work (p = 0.002), past skin care (p < 0.001), bathing frequency (p = 0.012), and bathing duration (p = 0.016).

In the multiple linear regression model (Table 4), the following associations were observed: SC hydration with clothing change frequency (β = 0.135, p = 0.029) and clothing type (β = − 0.139, p = 0.021); skin pH with clothing change frequency (β = − 0.137, p = 0.029); albumin level with age (β = − 0.130, p=0.044); and NGFβ with cumulative lifetime sun exposure at work (β = 0.145, p = 0.026) and bathing duration (β = − 0.151, p = 0.022).

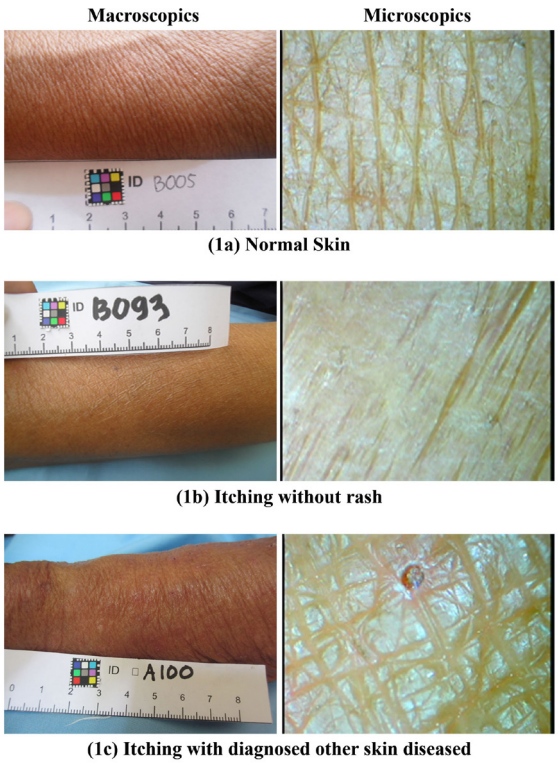

Figure 1. Macroscopic and microscopic photographs of forearm. (**a**). Normal skin: macroscopic photograph sample from participants without itching symptoms and skin disease signs. The microscopic photograph shows good skin structure, *i.e.*, skin with hair and sweat glands, clear skin triangle line, and no scales. (**b**). Itching without rash: macroscopic photograph sample from participants with itching symptoms but no skin disease signs, *i.e.*, no rash. The microscopic photograph shows bad skin structure, *i.e.*, skin without hair and sweat glands, and no skin triangle line, but no scales. (**c**). Itching with other skin diseases: microscopic photograph sample from participants with itching symptoms and skin disease signs. In this case, a dermatologist diagnosed eczema. The microscopic photograph shows bad skin structure, *i.e.*, skin without hair and sweat glands, unstructured skin triangle line, and with scales.

4. Discussion

This study evaluated albumin and NGFβ levels using skin blotting as a novel method of assessing itching without rash. This contributes to understanding the pathophysiology of itching in older adults. High albumin and NGFβ levels indicated disrupted skin barrier function and epidermal inflammation associated with itching without rash. Furthermore, the association of albumin and NGFβ with bathing emphasized the importance of skincare and hygiene in keeping the skin healthy and preventing itching in older adults.

The prevalence of itching in this study was 69.1%. The prevalence of itching in older adults was reported to be 6-42% in western countries (*1*). The relatively higher prevalence in this study was probably due to environmental conditions and racial skin property differences between western and Asian people (*26*). This study was conducted in a tropical region on the equator, with high temperatures and long sun exposure

Table 3. Comparison of skin properties between the itching without rash and normal skin groups (n = 276)

Items	Median (Range)		p-value
	Itching without rash (n = 83)	Normal skin (n = 193)	
SC hydration	66.0 (47.7-86.3)	76.3 (59.8-89.8)	0.007
Skin pH	5.0 (4.6-5.7)	4.8 (4.4-5.2)	0.012
Albumin (ng), n = 257	284.1 (206.2-536.4)	201.6 (145.8-265.3)	< 0.001
IL2 (pg)	337.8 (261.8-401.5)	334.2 (263.6-425.1)	0.654
NGFβ (ng)	276.0 (207.7-393.0)	209.1 (161.9-332.2)	< 0.001
TSLP (ng), n = 257	2.1 (1.7-2.4)	2.0 (1.8-2.3)	0.900

SC, Stratum corneum; IL2, Interleukin 2; NGFβ, Nerve growth factor β; TSLP, Thymic stromal lymphopoietin. Differences were compared using the Mann–Whitney U test.

Table 4. Multiple linear regression analysis predicting associated factors of skin properties of itching without rash

Items	SC hydration (n = 276)		Skin pH (n = 276)		Albumin (n = 257)		NGFβ (n = 276)	
	β	*p*	*β*	*p*	*β*	*p*	*β*	*p*
Age (years)	0.020	0.746	0.106	0.087	-0.130	0.044	-0.055	0.378
Sex	0.075	0.220	-0.084	0.177	0.061	0.341	0.031	0.620
Body mass index (kg/m^2)	0.077	0.193	-0.049	0.411	0.057	0.358	0.014	0.823
Barthel Index	-0.028	0.668	0.061	0.353	0.081	0.237	-0.024	0.716
Cumulative lifetime sun exposure at work (hours)	0.045	0.479	-0.013	0.837	0.118	0.076	0.145	0.026
Current skincare regimen	0.026	0.678	-0.067	0.282	-0.023	0.727	-0.039	0.533
Bathing frequency	0.109	0.094	-0.059	0.368	-0.134	0.051	-0.040	0.546
Bathing duration	0.098	0.129	-0.038	0.557	-0.054	0.423	-0.151	0.022
Clothing change frequency	0.135	0.029	-0.137	0.029	0.116	0.072	-0.003	0.958
Clothing type	-0.139	0.021	0.092	0.130	0.056	0.370	0.015	0.806
R^2	0.092		0.077		0.082		0.058	
Adjusted R^2	0.058		0.042		0.045		0.023	
P	0.004		0.017		0.018		0.095	

SC, Stratum corneum; NGFβ, Nerve growth factor β. Sex: 0 = Male, 1 = Female, Cumulative lifetime sun exposure at work (hours): 0 = ≤100,000 h, 1 = ≥ 100,001, Bathing frequency: 0 = ≤ 1 time/day, 1 = ≥ 2 times/day, Bathing duration: 0 = ≤ 5 minutes, 1 = > 5 minutes, Clothing change frequency: 0 = ≤ 1 time/day, 1 = ≥ 2 times/day, Clothing type: 0 = Long sleeves, 1 = Short sleeves. β: standardized partial regression coefficients; R2: coefficients of determination.

throughout the year. The residents have therefore experienced relatively long sun exposure, resulting in photoaging. Skin stimulated by sunlight releases histamine in epidermal cells (*10*), which may contribute significantly to skin inflammation and itching (*27*). More than half the cases of itchy skin in this study were diagnosed as itching without rash. Itching without rash shows no macroscopic abnormalities; therefore, caregivers overlook itching symptoms in older adults, especially if the affected individuals cannot report it themselves. Thus, these results emphasize the importance of an objective assessment tool for itching without rash in older adults.

Skin pH, SC hydration, and albumin levels in skin blot are indicators of skin barrier functionality (*17,28*). As they were all associated with itching without rash, they are candidate tools for its objective assessment. The skin barrier consists of sebum, a corneocyte lipid envelope, and tight junctions of the stratum granulosum. Sebum, the lipid-rich substance secreted from the sebaceous glands, forms a hydrophobic thin layer on the skin's surface. Skin commensal bacteria hydrolyze sebum and release free fatty acids. As a result, the skin surface is kept in an acidic state to prevent the growth of pathogenic microbes (*29*). The corneocyte lipid envelope consists of ceramide and fatty acids and forms a lamellar structure overlying the water and lipid layers to restrict water and electrolyte leakage and prevent irritant invasion (*30*). The tight junction in the stratum granulosum plays an important role in the selective permeability of substances, including albumin (*31*).

However, in this study, skin pH and SC hydration findings did not indicate impaired skin barrier function. These results were similar to those previously reported in healthy skin, although the results in the itching without rash group were significantly different from those in the normal skin group in this study (*28,32*). Lower SC hydration and higher skin pH significantly correlated with a low clothing change frequency and use of short sleeves. Skin with hair, scales, and sweat glands was significantly correlated with low cumulative lifetime sun exposure, doing skin care, low bathing frequency, and low clothing change frequency. These correlations suggested that stimulation with sweat, water, and sun exposure induced itching without rash (*33*). In contrast, skin blotting showed higher albumin levels in the itching without rash group than in the normal skin group, suggesting that this method could evaluate skin barrier function appropriately regardless of environmental conditions.

Invisible skin inflammatory status was evaluated by skin blotting using NGFβ, IL2, and TSLP levels. NGFβ is a marker for epidermal inflammation, while IL2 and TSLP are markers of Th1-type and Th2-type inflammation, respectively. A higher NGFβ level was significantly related to the presence of itching without rash, whereas no significant difference was found between itchy skin without rash and normal skin in IL2 and TSLP. NGFβ upregulates neuropeptides, especially substance P (SP) and calcitonin-gene-related peptide, in adult rat primary sensory neurons (*34*). Neuropeptides are involved in hypersensitivity itch sensation, and neurogenic inflammation also causes skin scratching (*35*). Furthermore, factors associated with a higher NGFβ level were bathing duration and cumulative lifetime sun exposure at work. These results suggest that itching without rash is induced by water exposure during hygiene care (aquagenic pruritus) (*36*) and sun exposure induces cutaneous inflammation (*35*). Hypo-osmotic shock by water exposure leads to cellular damage in the epidermis, and keratinocytes release NGFβ, inducing the elongation of C nerve fibers in the epidermis and resulting in hypersensitive itching (*18*,*37*). Our findings showed that the skin blotting examination of NGFβ is a

possible assessment tool for itching without rash.

In this study, the multiple linear regression model analysis revealed the association of skin properties of itching without rash with sun exposure, bathing frequency, bathing duration, and clothing change frequency. These findings implied the importance of skincare and hygiene care for preventing itching without rash. Future studies should be based on longitudinal designs to test the association direction and further potential presence of itching without rash.

Certain limitations of this study should be mentioned. First, this study was conducted in LTCs in a city without randomizing selection. Nevertheless, we assumed that public LTCs have the same standard of care, thereby reducing bias due to non-randomization. Second, for participants without itching on their forearm, we performed skin examinations only on the left forearm. In the previous study, we confirmed that normal skin on both forearms have similar skin properties (*7*,*14*).

In conclusion, the prevalence of itching was 69.1%, and approximately half these cases were diagnosed as itching without rash. Albumin and NGFβ in skin blotting, SC hydration, and skin pH were significantly correlated with itching without rash. The skin properties of pruritic skin were significantly correlated with skincare; hygiene care practices, including bathing and changing clothes; and aging and sun exposure. However, further studies are needed to reveal which skincare regimes and maximum duration of water exposure are compatible with hygiene care for older adults' skin. Measurement of albumin and NGFβ levels using skin blotting was identified as an objective and non-invasive assessment method. Thus, albumin and NGFβ are appropriate biomarkers for itching without rash.

Acknowledgements

We are grateful to Indonesian government for giving permission us in data collection at LTC. The author also expresses gratitude to all of participants who took part in this study.

Funding: This work was supported by Japan Society for the Promotion of Science KAKENHI Grant Numbers 15H05066; and the Indonesia Endowment Fund for Education (LPDP). Grant do not have involvement in study design, data collection, analysis, interpretation, and writing of this paper.

Conflict of Interest: Prof. Takeo Minematsu belong to a department that receives financial support from Saraya Corporation, however, this company did not have any concern in this study.

References

1. Weisshaar E, Dalgard F. Epidemiology of itch: adding to the burden of skin morbidity. Acta Derm Venereol. 2009; 89:339-350.
2. Cowdell F, Dyson J, Long J, Macleod U. Self-reported skin concerns: An epidemiological study of community-dwelling older people. Int J Older People Nurs. 2018; 13:e12195.
3. Hahnel E, Lichterfeld A, Blume-Peytavi U, Kottner J. The epidemiology of skin conditions in the aged: A systematic review. J Tissue Viability. 2017; 26:20-28.
4. Weisshaar E, Kallen U, Weiß M. "The itching hand" – important differential diagnoses and treatment. J Dtsch Dermatol Ges. 2013; 11:31-42.
5. National Clinical Guideline Centre (UK). The prevention and management of pressure ulcers in primary and secondary care. London; 2014.
6. Minematsu T, Horii M, Oe M, Sugama J, Mugita Y, Huang L, Nakagami G, Sanada H. Skin blotting: A noninvasive technique for evaluating physiological skin status. Adv Skin Wound Care. 2014; 27:272-279.
7. Sari DW, Minematsu T, Yoshida M, Noguchi-Watanabe M, Tomida S, Kitamura A, Abe M, Sanada H. Validity of skin blot examination for albumin and nerve growth factor β to detect itching of the skin in Indonesian older adults. J Tissue Viability. 2021; 30:42-50.
8. Golińska J, Sar-Pomian M, Rudnicka L. Haemorrhagic dots as a dermoscopic marker of itch severity in psoriasis. Australas J Dermatol. 2021; 62:e559-e562.
9. Murota H, Katayama I. Exacerbating factors of itch in atopic dermatitis. Allergol Int. 2017; 66:8-13.
10. Malaviya R, Morrison AR, Pentland AP. Histamine in human epidermal cells is induced by ultraviolet light injury. J Invest Dermatol. 1996; 106:785-789.
11. Garibyan L, Chiou AS, Elmariah SB. Advanced aging skin and itch: Addressing an unmet need. Dermatol Ther. 2013; 26:92-103.
12. Dickison P, Swain G, Peek J, Smith S. Itching for answers: Prevalence and severity of pruritus in psoriasis. Australas J Dermatol. 2017; 79:AB172.
13. Elke W, Uwe G, Jörg K, Masutaka F, Hidehisa S, Gil Y. Questionnaires to assess chronic itch: a consensus paper of the special interest group of the international forum on the study of itch. Acta Derm Venereol. 2012; 92:493-496.
14. Koyano Y, Nakagami G, Minematsu T, Sanada H. Reliability of the skin blotting method when used on the elderly. Int Wound J. 2018:807-813.
15. Ya-Xian Z, Suetake T, Tagami H. Number of cell layers of the stratum corneum in normal skin - relationship on the anatomical location an the body, age, sex and physical parameters. Arch Dermatol Res. 1999; 291:555-559.
16. Lambers H, Piessens S, Bloem A, Pronk H, Finkel P. Natural skin surface pH is on average below 5, which is beneficial for its resident flora. Int J Cosmet Sci. 2006; 28:359-370.
17. Tamai N, Minematsu T, Tsunokuni S, Aso K, Higashimura S, Nakagami G. Detection of albumin using skin blotting as a measure of skin barrier function. J Nurs Sci Eng. 2017; 4:116-120.
18. Kishi C, Minematsu T, Huang L, Mugita Y, Kitamura A, Nakagami G, Yamane T, Yoshida M, Noguchi H, Funakubo M, Mori T, Sanada H. Hypo-osmotic shock-induced subclinical inflammation of skin in a rat model of disrupted skin barrier function. Biol Res Nurs. 2015; 17:135-141.
19. Romagnani S. T-cell subsets (Th1 versus Th2). Ann Allergy Asthma Immunol. 2000; 85:9-18.

20. Ziegler SF, Roan F, Bell BD, Stoklasek TA, Kitajima M, Han H. The biology of thymic stromal lymphopoietin (TSLP). Adv Pharmacol. 2013; 66:129-155.
21. Nakai A, Minematsu T, Tamai N, Sugama J, Urai T, Sanada H. Prediction of healing in Category I pressure ulcers by skin blotting with plasminogen activator inhibitor 1, interleukin-1α, vascular endothelial growth factor C, and heat shock protein 90α: A pilot study. J Tissue Viability. 2019; S0965-206X:30051-2.
22. Minematsu T, Dai M, Tamai N, *et al*. Risk scoring tool for forearm skin tears in Japanese older adults: a prospective cohort study. J Tissue Viability. 2021; 30:155-160.
23. Karsten K. Geriatric depression scale in Indonesian version. *https://web.stanford.edu/~yesavage/GDS.html*.
24. Yesavage JA, Brink TL, Rose TL, Lum O, Huang V, Adey M, Leirer VO. Development and validation of a geriatric depression screening scale: A preliminary report. J Psychiatr Res. 1982; 17:37-49.
25. Yu CL, Li Y, Freedman DM, Fears TR, Kwok R, Chodick G, Alexander B, Kimlin MG, Kricker A, Armstrong BK, Linet MS. Assessment of lifetime cumulative sun exposure using a self- administered questionnaire: reliability of two approaches. Cancer Epidemiol Biomarkers Prev. 2009; 18:464-471.
26. Wesley NO, Maibach HI. Racial (ethnic) differences in skin properties: The objective data. Am J Clin Dermatol. 2003; 4:843-860.
27. Shim WS, Oh U. Histamine-induced itch and its relationship with pain. Mol Pain. 2008; 4:1-6.
28. Zaniboni M, Samorano L, Orfali R, Aoki V. Skin barrier in atopic dermatitis: Beyond filaggrin. An Bras Dermatol. 2016; 19:265-278.
29. du Plessis J, Stefaniak A, Eloff F, John S, Agner T, Chou TC, Nixon R, Steiner M, Franken A, Kudla I, Holness L. International guidelines for the *in vivo* assessment of skin properties in non-clinical settings: part 2. transepidermal water loss and skin hydration. Skin Res Technol. 2013; 19:265-278.
30. Grice EA, Segre JA. The skin microbiome. Nat Rev Microbiol. 2013; 9:244-253.
31. Elias PM, Gruber R, Crumrine D, Menon G, Williams ML, Wakefield JS, Holleran WM, Uchida Y. Formation and functions of the corneocyte lipid envelope (CLE). Biochim Biophys Acta. 2014; 1841:314-318.
32. Lizaka S, Takehara K, Sanada H. Validation of a portable device for measuring stratum corneum hydration. J Jpn Wound Ostomy Continence Manag. 2015; 19:33-39.
33. Rittie L, Fisher GJ. Natural and sun-induced aging of human skin. Cold Spring Harb Perspect Med. 2015; 5:1-14.
34. Verge VMK, Richardson PM, Wiesenfeld-Hallin Z, Hokfelt T. Differential influence of nerve growth-factor on neuropeptide expression *in vivo*: A novel role in peptide suppression in adult sensory neurons. J Neurosci. 1995; 15:2081-96.
35. Eschenfelder CC, Benrath J, Zimmermann M, Gillardon F. Involvement of substance-P in ultraviolet irradiation-induced inflammation in rat skin. Eur J Neurosci. 1995; 7:1520-1526.
36. Bircher AJ, Meier-Ruge W. Aquagenic pruritus: Water-induced activation of acetylcholinesterase. Arch Dermatol. 1988; 124:84-89.
37. Yamamoto H, Ikeda M, Okajima Y, Okajima M. Electrolytic-reduction ion water induces ceramide synthesis in human skin keratinocytes. Drug Discov Ther. 2021; 15:248-253.

Received December 14, 2022; Revised April 23, 2023; Accepted April 27, 2023.

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Released online in J-STAGE as advance publication May 11, 2023.